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ANALYSIS OF STRYCHNINE ABUSED AS OPIUM

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ABSTRACT

Recreational drugs, such as cocaine heroin opium are often adulterated with other pharmacological agents to either enhance or diminish the drug effects. A suspected sample of opium was referred to FSL for analysis. The HPTLC analysis gave positive indications for presence of morphine, one the main narcotic constituents of opium. The UV analysis and GLC analysis showed absence of morphine. Therefore, further GC-MS analysis was carried out for identification of the drug. The GC-MS studies indicated presence of strychnine and brucine. Further identification tests like HPTLC, UV, GLC were carried out using reference standards. Strychnine and brucine are the poisonous alkaloids obtained from the strychnine tree also known as nux vomica. Although it is best known as a poison, small doses of strychnine were used in medications as a stimulant, a laxative and as a treatment for other stomach ailments. Strychnine's stimulant effects also led to its use historically for enhancing performance in sports. A lethal dose was cited as 32 mg but people have been known to die from as little as 5 mg of strychnine. Adulteration of illicit drugs has become an epidemic health concern for drug users. Healthcare professionals need to be aware of this issue, so the patients can be treated in an effective and timely manner.

KEY WORDS

Strychnine, Brucine, HPTLC, UV, GLC, GC-MS.

INTRODUCTION

The strychnine tree also known as nux vomica is a major source of the highly poisonous alkaloids strychnine and brucine. Strychnine is very toxic alkaloid used as a pesticide particularly for killing small vertebrates such as bird and rodents. Strychnine causes violent convulsions due to a simultaneous stimulation of the motor or sensory ganglia of the spinal cord. During the convulsions, there is a rise in blood pressure and eventually death through asphyxia or sheer exhaustion. Strychnine is one of the most bitter substances known. It's taste is detectable in concentrations as low as 1 ppm Brucine closely resembles strychnine in its action, but is slightly less poisonous as it only causes paralysis of the peripheral motor nerves.

Morphine is the most prevalent and important alkaloid in opium. Regular use can lead to drug tolerance or physical dependence.

2.EXPERIMENTAL

All reagents used were of analytical grade, obtained from E.Merck India Ltd. Dragendorff's reagent used was freshly prepared. Reference Standard morphine & strychnine was obtained from Govt Opium & Alkaloids factories, Neemuch, M.P. Reference Standard brucine was obtained from E.Merck India Ltd.

2.1 Sample preparation

Reference standard solutions –

Strychnine – 1 mg/ ml in methanol was prepared.

Morphine – 1 mg /ml in methanol was prepared and filtered.

Brucine – 1 mg / ml in methanol was prepared and filtered.
Case sample solution – 25 mg / ml in methanol was prepared and filtered.

2.2. High Performance Thin Layer Chromatography
Equipment

HPTLC plates of 5 x 10 cm and 10x10 cm sizes having layer thickness 0.25 mm and precoated with $^{60}F_{254}$ silica gel E. Merck were used.

The plates were activated in oven at 110 ^{0} C for 10 minutes, then removed, cooled to room temperature and spotted by capillaries with standard drug solutions and samples under examination. The plate was then developed in solvent system ethyl acetate: methanol: ammonia in the ratio 17: 2: 1. The TLC chamber was presaturated with solvent system for about 10 minutes. The plate was developed until the solvent front travelled to 8 cm from the spotting. Then the plate was removed from chamber, air dried and sprayed with Dragendorff's reagent. Orange spots were obtained. The Rf values were noted. See Tables 1& 2.

2.3. Ultra violet spectrophotometry-
Equipment

UV system – Specord S600, attached with data station and Aspect Plus software was used for the analysis. Case sample solution in 0.1 N HCl was used for recording UV spectra.

2.4. Gas Liquid Chromatography–
Equipment

Gas Chromatograph –
GC System – Nucon model 5765 attached to data station with Nuchrom software was used for the analysis.

GC Operating conditions: (I)

Column: HP – 17, Capillary column, 30 m, 0.25µm film thickness.
Carrier gas: Nitrogen 30 ml / min
Detector: FID
Oven Temp: Initial 200 ^{0} C (hold time 1 min), final temp. 300 ^{0} C (final hold time 10 mins)
Ramp Rate: 20 ^{0} C / min.
Injector temp: 280 ^{0} C
Detector temp: 280 ^{0} C
2 µl of morphine reference standard solution injected and a gas chromatogram was obtained. 2µl of case sample was also injected into GC under conditions mentioned above.

GC Operating conditions: (II)

Column: HP – 17, Capillary column, 30 m, 0.25µm film thickness.
Carrier gas: Nitrogen 30 ml / min
Detector: FID
Oven Temp: Initial 260 ^{0} C (hold time 1 min), final temp. 300 ^{0} C (final hold time 10 mins)
Ramp Rate: 20 ^{0} C / min.
Injector temp: 280 ^{0} C
Detector temp: 280 ^{0} C
2 µl of of strychnine and brucine reference standard solutions injected and a gas chromatogram was obtained. 2µl of case sample was also injected into GC under conditions mentioned above.

2.5. Gas Chromatography – Mass Spectrometry
Equipment

GC-MS system – Trace MS plus from Thermofinnigan attached with data station, Xcalibure software was used for the analysis.
GC-MS operating conditions –
Column: HP – 5, capillary column.
Carrier gas: Helium.
Oven Temp: Initial – 180 ^{0} C (hold time 2.0 min)
Final – 250 ^{0} C (hold time 20 min)
Ramp rate – 15 ^{0} C / min.
Injector temp: 220 ^{0} C.
MS Conditions –
Ionization mode: EI +
Source Temp: 200 ^{0} C
Interface Temp: 180 ^{0} C
Mass Range: 10.00 – 550.00
1 µl of case sample and standard reference samples in methanol were injected.

3.RESULTS AND DISCUSSIONS

The test procedure for opium involves colour tests, ultra-violet spectrometry (UV), high performance thin layer chromatography (HPTLC), gas liquid chromatography(GLC), gas chromatography mass spectrometry(GC-MS) etc.

The case samples respond negative for colour tests ie the Marquis test[1]. The colour of case sample by marquis reagent is orange instead of violet.

UV spectrum in 0.1N aqueous acid is at 254 nm, 264nm and 300 nm. Since the UV for morphine in 0.1 N aqueous

acid is 285 nm, the UV technique failed to show the presence of morphine in case sample. Since the case sample is not in pure state there are limitations on UV technique. Because of interference of impurities in UV analysis, presence or absence of morphine in case sample cannot be confirmed. Therefore, further confirmation was required. UV spectrum of case sample is shown in Fig 1.

In HPTLC analysis[2] methanol solution of sample and reference standard of morphine were used. The precoated and preheated silica plates were used for analysis. The sample solution as well as morphine solution were spotted on TLC plates. The plates were kept in solvent chamber containing solvent system. The solvent system was allowed to migrate up to 10cm distance by capillary action. As the solvent migrates the components get separated. The distance traveled by the solvent and the distance traveled by separated components was measured and thereby the Rf values were calculated. The HPTLC pattern of case sample showed two spots one of which was almost tallied with that of morphine. The Rf values by thin layer chromatography (TLC) analysis, of morphine was 0.56 and that of case samples were 0.55 & 0.73. The Rf values of morphine almost matched with Rf values of case samples, but one additional spot at Rf 0.73 was observed in case sample. Thus, the TLC analysis indicated presence of morphine in case samples as shown in Fig 2.

Gas Chromatogram of case sample showed two peaks at the retention time 1.94 min. and 2.77 mins. Gas chromatogram of morphine showed the peak at retention time 6.31 mins. The GLC analysis of case sample indicated absence of morphine and presence of two additional peaks at the retention times 1.94 min. and 2.77 mins. as shown in Fig 3 & 4.

By GLC techniques the absence of morphine in case sample was confirmed. But further identification of case samples was needed. For this purpose, the unique full proof finger printing technique GC-MS[3] was applied.

GC-MS is a unique, reliable and full proof tool for identification purpose. GC-MS is recognized as one of the efficient analytical techniques and gives the highest degree of specificity in analysis. In GC-MS, a gas chromatograph is interfaced with mass selective detector, (MSD) when the sample is to be analysed is injected into the GC injection port it travels through the column when the different components of the sample are separated. The sepearted components then directed into the ionization chamber of mass detector, where they are bombarded by an electron beam. The high-energy electrons impact the separated component molecules. The resulting spectrum of each component is typically complex with large number of mass fragments. The components are then ionized and positively charged. This ionization results in fragmentation process. The molecular fragments traverse into magnetic field where they are separated according to their masses. A unique fragmentation pattern is obtained for the substance that is analysed.

The GC-MS results indicated presence of strychnine and brucine beyond the doubt. The typical TIC of the sample is shown in Fig 5. The library search reports of strychnine and brucine are shown in Fig 6 & Fig7 respectively.

The mass spectra of case sample exactly tallied with that in library of strychnine & brucine. The presence of strychnine & brucine. in case sample was further confirmed by HPTLC and GLC analysis. TLC analysis was carried out again with strychnine, brucine. & morphine as reference standards. The Rf values obtained by TLC analysis are listed in Table 2. The Rf value of strychnine was 0.73, that of brucine. was 0.55. & that of morphine was 0.56. The Rf values of case samples were 0.73 & 0.55 matching with that of strychnine & brucine. The thin layer chromatogram is shown in Fig 8.

The GLC analysis was carried out. The results indicated presence of Stychnine and brucine.

Table1: Rf values obtained by TLC analysis.

Sr.No.	Ref.Stds/samples	Rf values
1	Morphine	0.56
2	Case sample	0.55, 0.73

Table2: Rf values obtained by TLC analysis.

Sr.No.	Ref.Stds/samples	Rf values
1	Strychnine	0.73
2	Morphine	0.56
3	Brucine	0.55
4	Case sample	0.55, 0.73

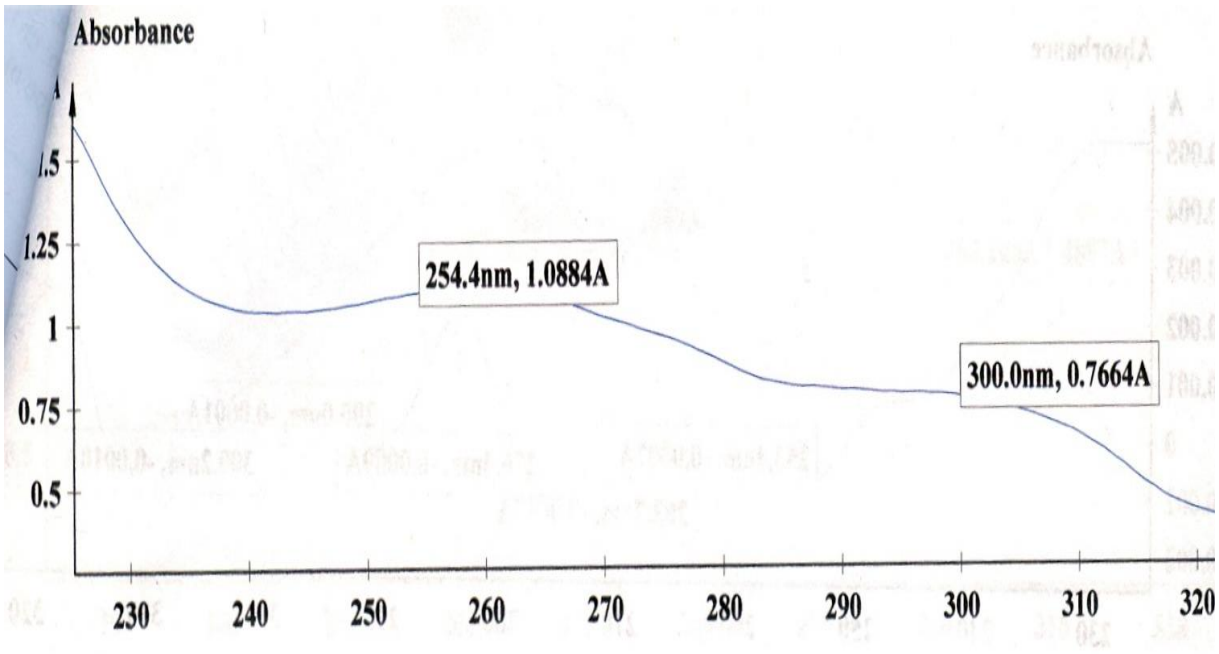


Figure-1: UV spectrum of case sample

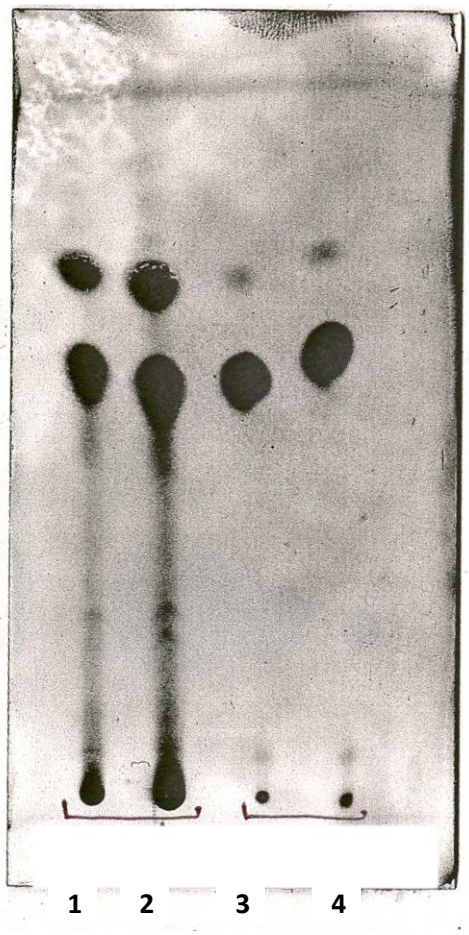


Figure-2:Thin Layer chromatogram

Track nos.1 & 2 –case samples. **Track Nos. 3 & 4- Morphine.**

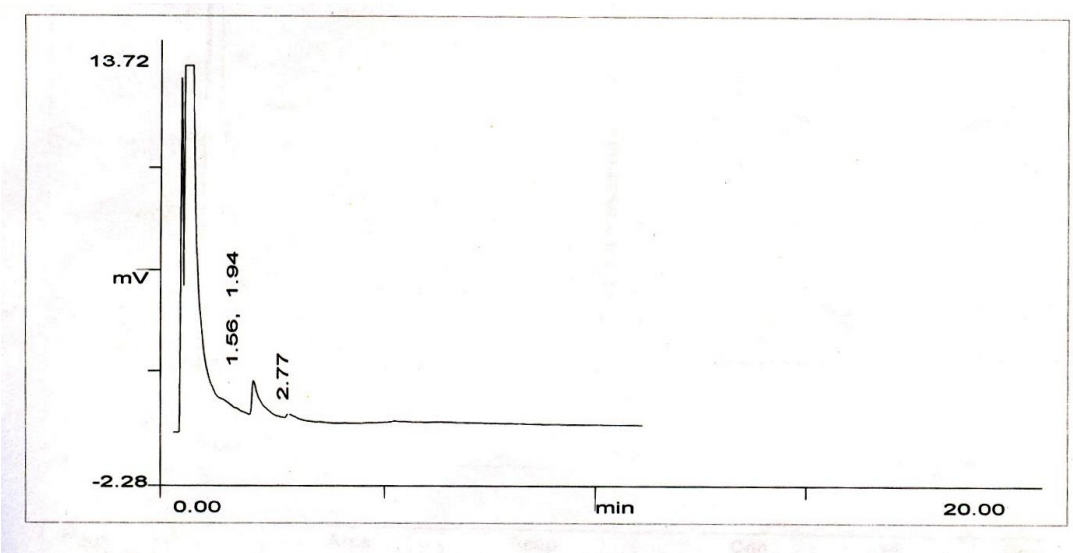


Figure-3: Gas chromatogram of case sample

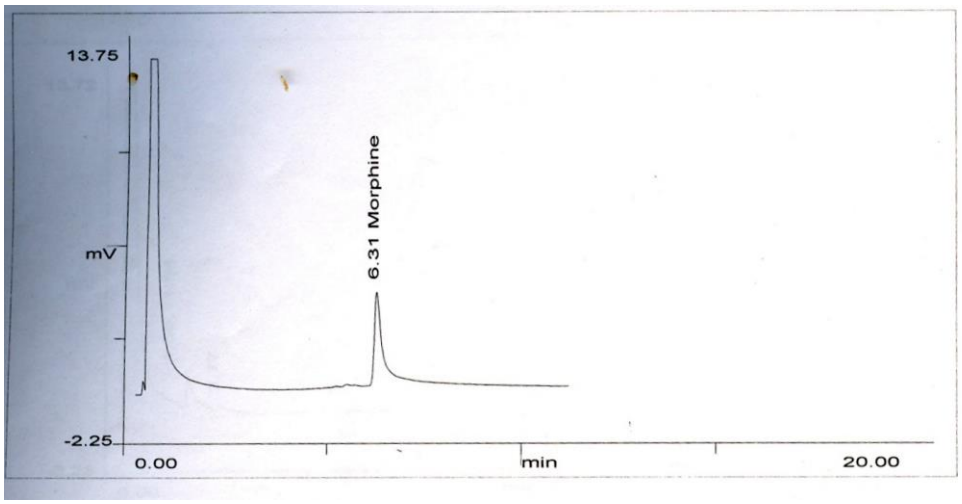


Figure-4: Gas chromatogram of ref.std. Morphine

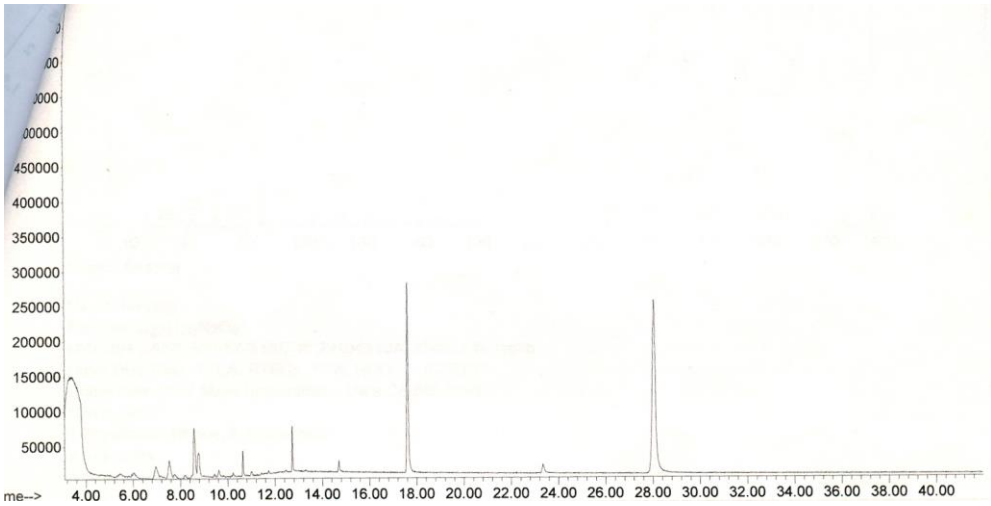


Figure-5 : GC-MS TIC of case sample.

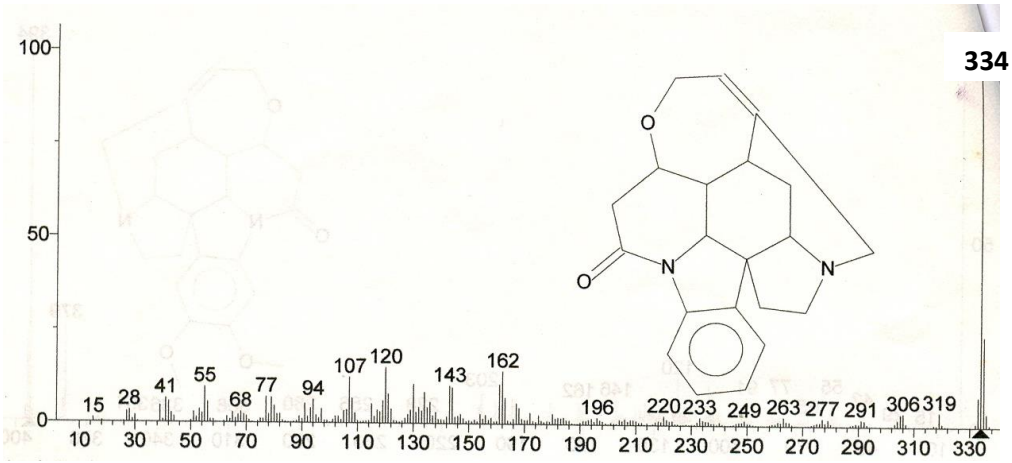


Figure-6 : Library search report of Strychnine

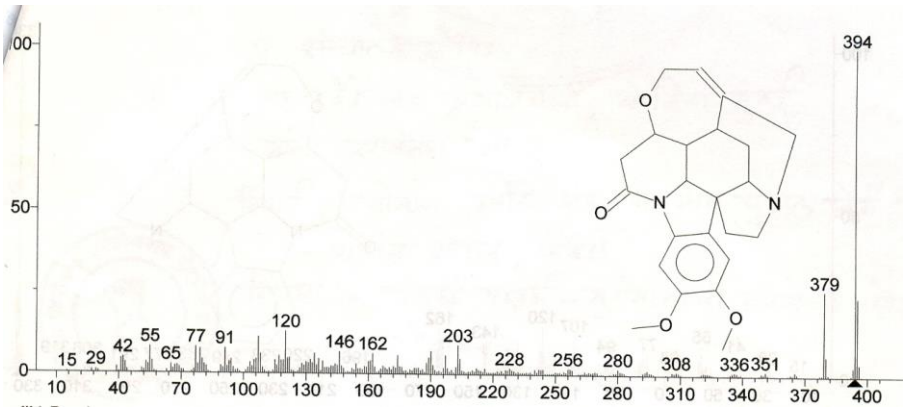
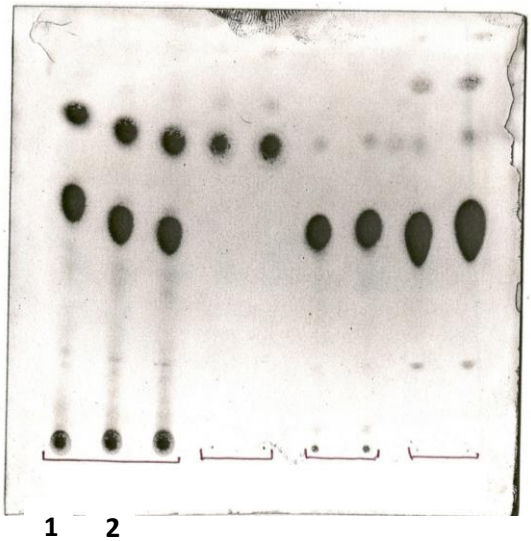


Figure-7 : Library search report of Brucine



1 2

Track nos.1, 2 & 3 –case samples. **Track Nos. 4 & 5- Strychnine**

Track nos.6 & 7 - Brucine **Track nos. 8 & 9 -Morphine**

Figure-8

4. Conclusion

Clandestine operators have safely introduced strychnine & brucine, in illicit market in place of opium. It helps them to escape easily from legal scrutiny / network. Healthcare professionals need to be aware of this issue, so the patients can be treated in an effective and timely manner.

5. References

1. 'Clarke's Isolation & Identification of Drugs', 2nd Ed. The pharmaceutical press1986, pg 746-747, 617-618.
2. 'Working Procedure Manual on Narcotics' published by DFS, MHA, New Delhi, 2005.
3. Drug Abuse Hand book by Editor in Chief Steven B. Karch.

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